ELabELN | Template Use Case White Paper

Flow Cytometry Experiment Template

Structured Documentation for Multicolor Immunophenotyping — From PBMC Isolation Through Compensated Gating and Population-Frequency Export

APPLIES TO

- Academic and life sciences research laboratories
- Biotechnology and cell biology R&D groups
- Diagnostics, clinical R&D, and immunology / immune-monitoring laboratories
- Contract research organizations (CROs)

RELEVANT COMPLIANCE & INTEGRITY FRAMEWORKS

- FDA 21 CFR Part 11 — Electronic records and signatures
- Good Clinical Practice (GCP) — Clinical cohort and sample documentation
- EU Annex 11 & GAMP 5 — Computerized systems and software validation
- NIH / NSF reproducibility and data-management requirements

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1. Executive Summary

A flow cytometry result — the percentage of cells expressing a marker, the ratio of one population to another — is only as trustworthy as the panel, the compensation, and the gating strategy that produced it. A multicolor panel is a small experiment in itself: each antibody carries a fluorochrome, a lot, and a titer; spectral overlap between fluorochromes must be compensated with single-color controls; and the final population frequencies depend on where the gates were drawn. Change an antibody lot, skip a compensation control, or shift a gate, and the numbers move — often without any obvious sign. Because flow results are routinely compared across samples, across a cohort, and across months, the reproducibility of every frequency depends on whether the panel, the acquisition settings, the compensation, and the gating were captured in a structured, comparable form. On a printout or in a lab notebook, they are not.

The ELabELN Flow Cytometry Experiment Template solves this by providing a pre-built, fully structured panel record that loads from the template library in a single click. It ships with a 10-step protocol, validated antibody-panel references, pre-wired compensation and gating fields, and typed inputs for sample source, fluorochrome panel, voltages, total events, and gated population percentages. Antibody aliquots, the viability dye, and titrated working dilutions link to the ELabELN inventory, and the tamper-evident audit trail captures every panel composition, every compensation run, and every gated frequency.

This white paper describes what the template contains, the immunophenotyping use case it is designed to support, the upstream and downstream workflows it connects to, the benefits it delivers to the scientist, and how a laboratory can deploy it and begin running panels within days.

> **KEY BENEFIT**
> Labs that adopt the flow template stop rebuilding the panel record from scratch each run, capture antibody-lot and titer provenance automatically, and gain cross-run QC — query every run that used a given panel version or antibody lot and compare population frequencies side by side — in a record built to support panel-version documentation and 21 CFR Part 11 / GCP review.

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2. What Is Flow Cytometry, and Why Does Documentation Matter?

2.1 The Technique

Flow cytometry measures the physical and fluorescent properties of individual cells as they pass single-file through one or more laser beams. Cells are stained with a panel of antibodies, each conjugated to a distinct fluorochrome that marks a specific surface or intracellular protein; as each cell is interrogated, the cytometer records its forward and side scatter (size and granularity) and the intensity of each fluorochrome. Because fluorochrome emission spectra overlap, single-color compensation controls are run to mathematically correct spillover between detectors, and fluorescence-minus-one (FMO) controls are used to set the boundary between positive and negative for critical markers. The corrected data are then analyzed by drawing a hierarchy of gates — successive subsets that isolate the population of interest — from which the frequency of each population is calculated.

This is the standard method for immunophenotyping, intracellular cytokine staining, cell-cycle analysis, and apoptosis assays. Its power is its multiparameter, single-cell resolution; its fragility is that the final frequencies rest on a chain of choices — which panel, which titers, which voltages, whether compensation was run that day, and where the gates were placed. A five-color T cell panel reporting a CD4:CD8 ratio is only meaningful if that entire chain is documented.

Because flow frequencies are compared across samples and cohorts, **the panel composition, the acquisition settings, the compensation status, and the gating strategy — with the antibody lots and titers behind them — are what make one run comparable to another, which is exactly why they must be captured as structured data rather than a printed plot.**

2.2 The Documentation Problem

Recording flow experiments in a paper notebook or ad-hoc spreadsheet creates a set of interconnected risks:

- The panel — fluorochrome, marker, lot, and titer for each antibody — is rewritten by hand each run, so a lot or titer change that shifts a population cannot be traced.
- Compensation status (whether single-color controls were run that day, and the method used) is not recorded, so a questionable result cannot be checked against its compensation.
- Voltages, total events, and gated frequencies live on a printout, where they cannot be queried or compared across runs.
- The FCS files and the FlowJo workspace are disconnected from the panel and acquisition record, breaking the link between raw data and reported frequencies.
- There is no timestamped, attributed record of who ran which panel and when — a baseline requirement for shared cohorts and regulated work.
- Prior runs of the same panel cannot be retrieved to compare, so cross-run and cohort consistency is invisible.

These are the gaps that undermine flow reproducibility, and they are the gaps this template was designed to close.

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3. Template Overview: What Ships with the Template

The ELabELN Flow Cytometry Experiment Template is a pre-populated experiment record available to all ELabELN subscribers directly from the template library. It ships configured for a five-color T cell phenotyping panel on peripheral blood mononuclear cells (PBMCs), with a 10-step protocol covering PBMC isolation through compensated gating and population-frequency export. The default configuration assumes a BD FACSAria III or similar cytometer with 488 nm and 405 nm lasers; a CD3-BV421 / CD4-FITC / CD8-PE / CD45-PerCP / Live-Dead-APC panel; single-color compensation controls; FMO controls for marker boundaries; and 100,000 collected events per sample. Every section is editable — the configuration is a tested baseline, not a locked format.

3.1 Template Sections at a Glance

The template populates the existing ELabELN experiment sections your lab already works in. Your team edits a working baseline instead of rebuilding the panel record from scratch every experiment.

Section	What it pre-populates
Main Text	Panel design notes, sample-source notes, and the expected population-frequency ranges, authored in the TinyMCE rich-text editor and linked to the upstream PBMC isolation.
Extra Fields	Twelve structured, typed fields grouped as Sample, Panel, and Acquisition & Gating — sample source, isolation method, viability before stain, fluorochrome panel composition, antibody titers, compensation status, total events, and gated percentages for major populations. Typed values make panel composition and acquisition parameters queryable across runs.
Steps	A 10-step workflow checklist covering PBMC isolation through gated-frequency export. Each tick is timestamped to the audit trail, and the cytometer run is auto-logged.
Compounds	The antibody panel (CD3, CD4, CD8, CD45), viability dye, FACS buffer, and Ficoll pre-linked from the compound database, with antibody lots and titrated working dilutions carried in automatically.
Links & Resources	Pre-wired to the lab's PBMC Flow SOP v2.7, the panel validation document, and the FlowJo workspace for the study.
Storage & Files	4 °C antibody aliquot storage and frozen PBMC vials, plus labelled attachment slots for FCS exports and FlowJo workspace (WSP) files.

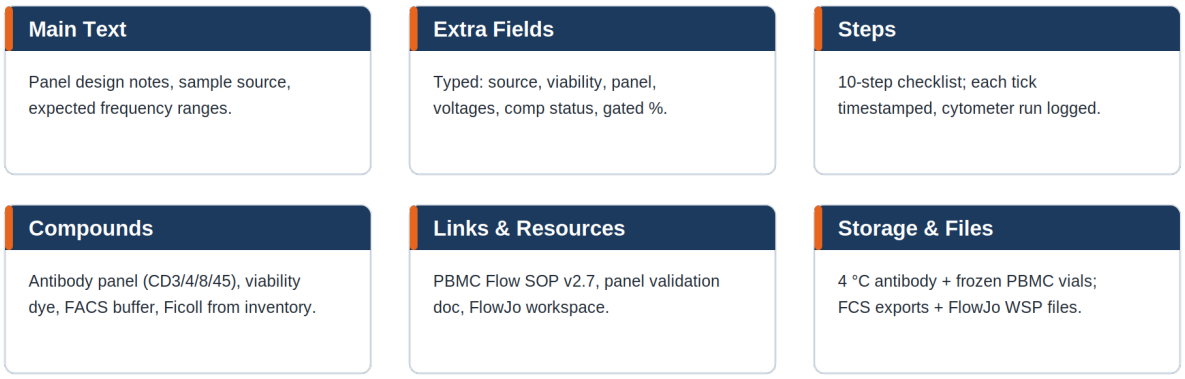
Main Text
Panel design notes, sample source, expected frequency ranges.
Extra Fields
Typed: source, viability, panel, voltages, comp status, gated %.
Steps
10-step checklist; each tick timestamped, cytometer run logged.
Compounds
Antibody panel (CD3/4/8/45), viability dye, FACS buffer, Ficoll from inventory.
Links & Resources
PBMC Flow SOP v2.7, panel validation doc, FlowJo workspace.
Storage & Files
4 °C antibody + frozen PBMC vials; FCS exports + FlowJo WSP files.

Figure 1. The six ELabELN experiment sections pre-populated by the flow cytometry template.

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3.2 The 10-Step Protocol

The workflow checklist is the operational spine of the template. Each step maps to a discrete bench or instrument action and writes a timestamped, user-attributed entry to the audit trail when checked off. The cytometer run is auto-logged, and the final gating and export steps are performed in the lab's analysis tool, with the resulting frequencies recorded as typed fields.

Step	Action	What it captures
1	Isolate PBMCs (Ficoll)	Sample source; isolation method
2	Count & check viability	Viability before stain
3	Stain (antibody panel)	Fluorochrome panel; antibody titers
4	Wash	Wash step
5	Add viability dye	Viability dye
6	Run compensation controls	Single-color controls; compensation status
7	Acquire (cytometer, 100k events)	Voltages; total events (run auto-logged)
8	Compensate	Compensation method; spillover adjustments
9	Gate (FlowJo)	Gating strategy (external tool)
10	Export frequencies & report	Gated % per population; CD4:CD8; e-signature

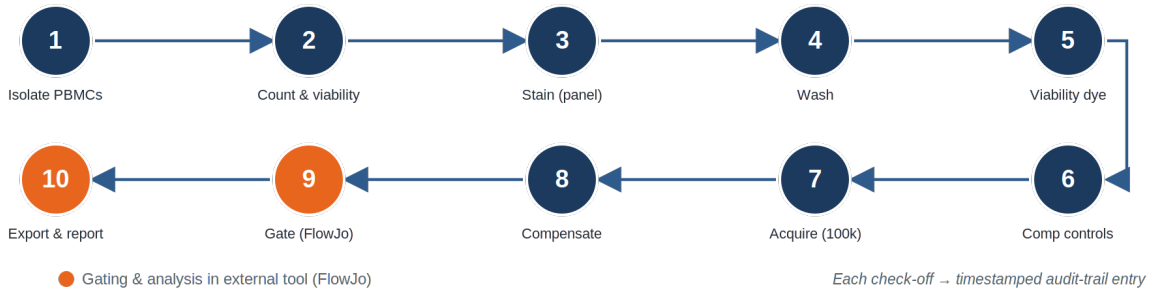

Figure 2. The 10-step flow cytometry workflow — orange steps are the gating and analysis performed in an external tool (FlowJo); every check-off writes a timestamped entry to the ELabELN audit trail.

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4. The Use Case: When and How the Template Is Used

The template is intended as a tested baseline for academic and life sciences labs running flow-based cell analysis. It is configured around a recognizable reference — a five-color T cell immunophenotyping panel on PBMCs — but the scenarios it serves span the whole flow bench.

4.1 Immunophenotyping (Primary Use Case)

The core scenario: identifying and quantifying cell populations by their surface markers. The template captures the panel, the compensation status, the acquisition settings, and the gated frequencies in one structured record, so a CD4:CD8 ratio or a rare-population frequency is fully reconstructable — which panel, which lots, which gates — rather than a number on a printed plot.

4.2 Intracellular Cytokine Staining

Cytokine staining adds fixation, permeabilization, and intracellular antibodies to the panel. The template's panel and acquisition fields extend directly to these markers, and the FMO-control linkage documents how positive boundaries were set for activation markers.

4.3 Cell-Cycle Analysis

Cell-cycle work (DNA-content staining) uses the same acquisition and gating structure with a different readout. Swap the panel for a DNA dye, and the acquisition and gated-population fields capture the cycle-phase distribution.

4.4 Apoptosis Assays

Apoptosis panels (Annexin V / viability dye) fit the same structure, with the gated frequencies capturing live, early-apoptotic, and late-apoptotic fractions in a comparable, queryable form.

4.5 Cohort Studies and Panel-Version QC

Because panel composition, antibody lots, and gated frequencies are captured together, the template doubles as a running QC record for a panel across a cohort. A lab can pull every run that used a specific panel version or antibody lot and compare population frequencies and compensation side by side — the objective basis for validating a new lot or investigating drift across a long study.

4.6 Multi-User, Shared-Cohort, and CRO Settings

Because every ELabELN edition includes unlimited users, the PI, postdocs, students, and visiting collaborators can all run panels from the same template, and the pre-wired SOP enforces one consistent procedure across a shared cohort. Granular permissions let the PI decide who may edit flow runs versus only review them — the basis a CRO needs to standardize panel documentation across client studies.

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5. Dependencies and Connected Workflows

The flow cytometry template does not exist in isolation. It sits inside a network of connected workflows — upstream, parallel, external, and downstream — that give the panel record its full scientific and provenance value.

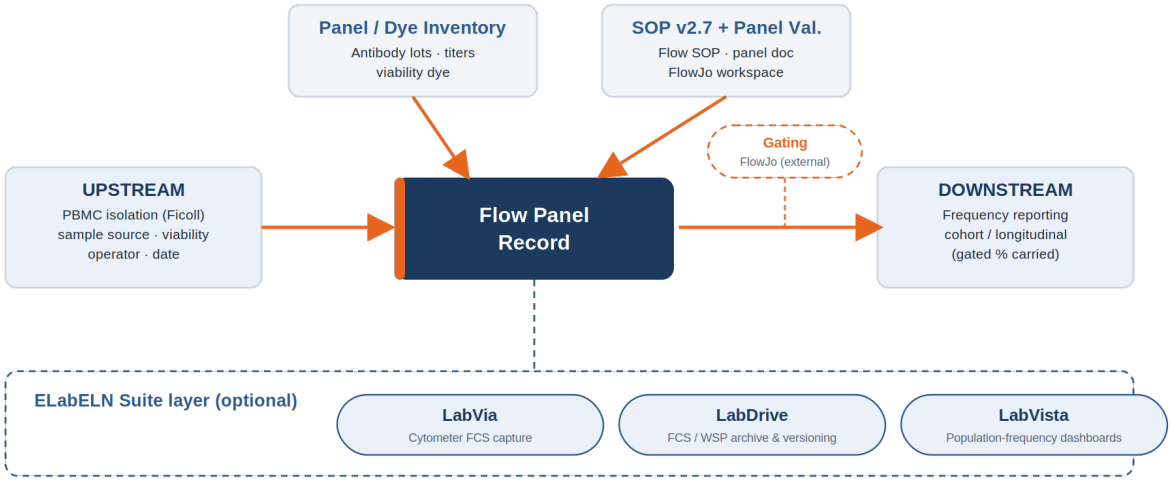

Figure 3. The template connects an upstream PBMC isolation, parallel inventory and SOP/panel-validation systems, external FlowJo gating, and downstream frequency reporting and cohort analysis.

5.1 Upstream: PBMC Isolation and Sample Source

Every panel begins with a cell suspension — PBMCs isolated by Ficoll, whole blood, dissociated tumor, or a cell line. The template links to the upstream isolation record, so opening a panel surfaces the sample source, the isolation method, the viability before staining, and the operator and date, anchoring every frequency to the material it came from.

5.2 Parallel: Antibody Panel and Viability-Dye Inventory

The Compounds section links the antibody panel (CD3, CD4, CD8, CD45), the viability dye, FACS buffer, and Ficoll directly to the ELabELN inventory. Because antibody lot and titer drive staining intensity, this traceability is decisive. Through the link the record automatically carries:

- **Antibody lots, shown inline**, so a lot change that shifts a population can be traced.
- **Titrated working dilutions**, so the exact staining conditions are on the record and reproducible run to run.
- **Expiry and stock levels**, with low-stock warnings before a run, so a panel is not started against a depleted or expired reagent.

Barcode and QR scanning can populate these links from the physical vial, keeping panel capture effortless.

5.3 Parallel: SOPs, Panel Validation, and FMO Controls

The Links section connects the run to the lab's version-controlled PBMC Flow SOP (v2.7 by default), the panel validation document, and the FlowJo workspace for the study. FMO (fluorescence-minus-one) control samples are recorded as separate experiments linked to the

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panel run, with a field for whether FMO boundaries were used and for which markers — supporting the typical workflow where activation or rare-population markers need an FMO to set the positive-gate threshold.

5.4 External: The Cytometer and FlowJo Gating

The cytometer produces the raw FCS data, and the gating analysis is performed in FlowJo. ELabELN is the system of record around that analysis: the panel, voltages, total events, and compensation status are captured as typed fields; the gated population frequencies (CD3+, CD3+CD4+, CD3+CD8+, CD4:CD8 ratio) live in the spreadsheet editor; and the raw FCS export and the FlowJo workspace attach to the record. The compensation matrix is recorded as part of the acquisition metadata — whether single-color controls were run the same day, the method used (auto or manual), and any spillover adjustments applied. The panel, the raw data, and the reported frequencies stay together in one record.

5.5 Downstream: Frequency Reporting and Cohort Analysis

The gated frequencies feed reporting and, because they and the panel parameters are typed and queryable, cross-run and longitudinal cohort analysis. A study spanning many samples can be assembled and reviewed for consistency directly from the structured records.

5.6 The ELabELN Suite: Instruments and Dashboards

Everything above is available in ELabELN Standard. Labs on the ELabELN Suite gain three layers that connect to the same record: LabVia captures FCS data directly from the cytometer; LabDrive adds metadata search, versioning, and role-based access across FCS and workspace archives; and LabVista builds population-frequency dashboards on the structured panel and acquisition metadata. The same audit trail and data-integrity controls span all layers.

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6. How the Scientist Benefits

The template is built to deliver value at the bench, not just to the quality system. The benefits compound with every panel a lab runs.

6.1 Start From a Tested Baseline, Not a Blank Panel

The scaffold — protocol, panel, typed fields, antibody and dye links, SOP and validation document — is already built. The scientist edits a working configuration to match the panel and sample they actually run, rather than rebuilding the panel record from scratch every experiment. The first run is faster, and every subsequent one is consistent with it.

6.2 Document by Doing

Checking off each of the 10 steps as it happens produces a contemporaneous, timestamped record as a by-product of the experiment, and the cytometer run is auto-logged. There is no separate write-up to reconstruct later.

6.3 Never Lose Panel or Titer Provenance Again

Because the antibodies and viability dye are linked to inventory, their lots and titrated dilutions are captured automatically and permanently on every run. When a population shifts, the panel and lot history is on the record — closing the most common flow troubleshooting gap by default.

6.4 Cross-Run and Cohort QC You Can Query

Typed panel, acquisition, and frequency fields make QC queryable. A scientist can pull every run that used a panel version or antibody lot, compare population frequencies and compensation at a glance, and catch drift across a cohort early — instead of discovering an inconsistency after a study is complete.

6.5 Produce Audit-Ready Records for Cohort and Regulated Work

The tamper-evident audit trail, version-controlled SOP linkage, and electronic signatures mean the record that results from normal use is already structured to support panel-version documentation, multi-PI collaboration on shared cohorts, and FDA 21 CFR Part 11 and GCP review when the lab's quality system requires it.

> **AT THE BENCH, IN PRACTICE**
> A researcher opens the template, sets the panel and sample source to match today's run, scans the antibody vials to capture their lots and titers, and checks off each step as staining and acquisition proceed. The cytometer run is auto-logged with voltages and event count; compensation status is recorded; and after gating in FlowJo, the population frequencies go into typed fields with the FCS and workspace attached. Months later, comparing a cohort, the PI queries every run that used that panel version and sees the frequencies line up — or spots the sample that drifted.

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7. The Panel, the Gating Strategy, and Population Frequencies

7.1 The Default Reference: A Five-Color T Cell Panel

The template ships configured for a five-color T cell phenotyping panel on PBMCs — CD3-BV421, CD4-FITC, CD8-PE, CD45-PerCP, and a Live/Dead-APC viability dye — acquired on a BD FACSAria III or similar cytometer with 488 nm and 405 nm lasers, with single-color compensation controls, FMO controls for marker boundaries, and 100,000 events per sample. The gating strategy proceeds from scatter (lymphocytes), through singlets and live cells, to CD3+ T cells, and finally to a CD4-versus-CD8 quadrant that yields the CD4:CD8 ratio. This concrete reference demonstrates the full panel-and-gating workflow out of the box; swap the panel, sample source, or gating strategy to match what your lab runs.

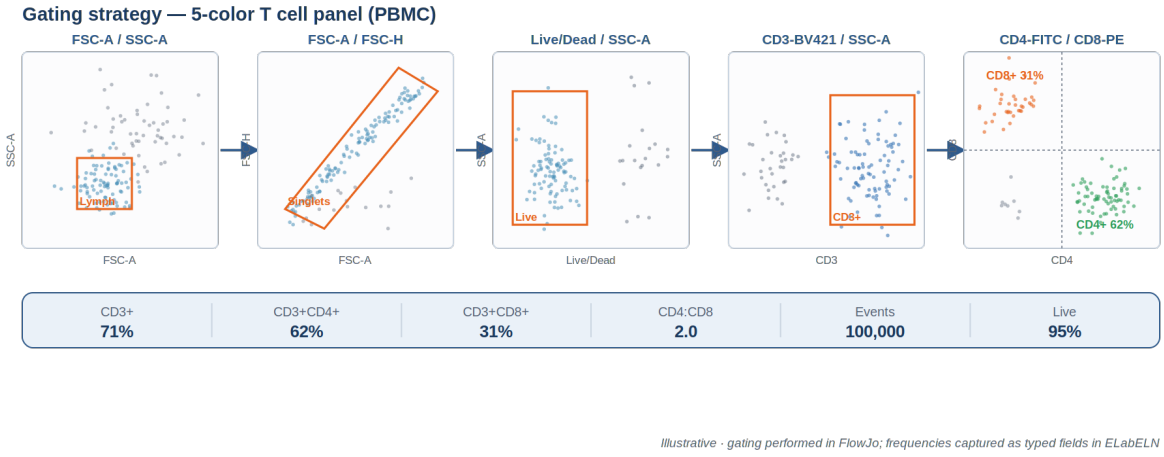

Figure 4. The reference gating strategy — from scatter and singlets through live and CD3+ gating to a CD4/CD8 quadrant, with population frequencies summarized. Illustrative; gating is performed in FlowJo and the frequencies are captured as typed fields in ELabELN.

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7.2 How the Template Handles the Panel, Compensation, and Frequencies

The panel composition, antibody titers, voltages, total events, and compensation status are captured as typed fields, and the gated population frequencies (CD3+, CD3+CD4+, CD3+CD8+, CD4:CD8 ratio) live in the spreadsheet editor. Gating itself is performed in FlowJo, and the raw FCS export and FlowJo workspace attach to the record, preserving the full audit trail for the analysis. Because the panel and frequencies are structured and queryable, cross-run and cohort analysis is straightforward: query every run that used a specific panel version or antibody lot and review frequencies and compensation side by side, rather than treating each run as an isolated result.

Element	Default configuration	Editable to
Panel	5-color (CD3/CD4/CD8/CD45/Live-Dead)	3-color through 18-color spectral
Sample source	PBMC (Ficoll)	Whole blood, dissociated tumor, cell line
Viability dye	Live/Dead-APC	Live/Dead Near-IR, Zombie Aqua, 7-AAD, PI
Cytometer	BD FACSAria III (488 / 405 nm)	Any cytometer / laser configuration
Analysis	Gating in FlowJo; typed frequencies	Any gating strategy

Figure 5. The template's default configuration — fully editable for any panel, sample source, cytometer, or gating strategy.

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8. Configuring the Template for Your Panel

Adapting the template to a different panel or sample is a matter of editing the relevant sections — not rebuilding the structure.

8.1 Swap the Fluorochrome Panel

Replace the panel with your own, from 3-color through 18-color spectral. If the antibodies already exist in the ELabELN compound database, simply re-link them; their lots and titrated dilutions flow in automatically.

8.2 Change the Sample Source and Viability Dye

Switch the sample source (PBMC, whole blood, dissociated tumor, cell line) and the viability dye (Live/Dead Near-IR, Zombie Aqua, 7-AAD, PI) to match your assay. The Sample, Panel, and Acquisition & Gating field groups apply across all of them.

8.3 Change the Cytometer, Lasers, and Gating Strategy

Edit the cytometer model and laser configuration to match your instrument, and adjust the gating strategy for your populations — the gated-frequency fields accommodate whatever hierarchy your analysis uses.

8.4 Save a Custom Version

Once configured, save your edits as a private template scoped to your lab, or publish back to the ELabELN template library. Every subsequent run of that panel opens from your configured version, and separate versions can be maintained for different panels, sample types, or regulatory contexts.

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9. Getting Started

ELabELN Standard is a cloud-hosted SaaS deployment typically live within one to two business days of account creation. There is no hardware to buy, no IT project, and no per-seat licensing.

1. **Start an ELabELN account.** Visit elabeln.com/get-started. ELabELN Standard runs on LabLynx's managed cloud infrastructure.
2. **Provision your instance.** LabLynx provisions the instance within one to two business days; you receive a URL, admin credentials, and onboarding documentation.
3. **Load the flow cytometry template.** From the template library, select the flow cytometry template and click “Use This Template.” An editable instance opens immediately.
4. **Add your antibodies and reagents to the inventory.** Enter your antibody panel, viability dye, FACS buffer, and Ficoll with lot numbers, titrated dilutions, and 4 °C aliquot locations. This is a one-time setup step.
5. **Link your SOP, panel validation, and FlowJo workspace.** Upload your flow SOP and panel validation document and connect them, along with the study's FlowJo workspace, to the template's Links section.
6. **Customize the template for your panel.** Edit the fluorochrome panel, sample source, viability dye, cytometer and lasers, and gating strategy, then save as a private team version.
7. **Run your first panel.** Open your saved template, complete the 10-step checklist, record the acquisition and compensation metadata and gated frequencies, attach the FCS and workspace files, and have the PI sign off electronically.

TYPICAL TIMELINE

Day 1: Account requested at elabeln.com/get-started. Day 2–3: Instance provisioned; antibody/dye inventory populated; SOP, panel validation, and FlowJo workspace linked. Day 3–4: Template configured and saved as a private version for your panel. Day 4–5: First panel documented in ELabELN with acquisition, compensation, and gated frequencies captured and an e-signature.

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9.1 ELabELN Standard vs. ELabELN Suite

Capability	Standard	Suite
Flow cytometry template access	Included	Included
Unlimited users	Included	Included
Audit trail and e-signatures	Included	Included
Antibody / dye inventory with lot & titer tracking	Included	Included
SOP, panel validation & FlowJo workspace linking	Included	Included
FCS + FlowJo workspace (WSP) attachments	Included	Included
LabVia direct cytometer FCS capture	—	Suite layer
LabDrive FCS / WSP archive search & versioning	—	Suite layer
LabVista population-frequency dashboards	—	Suite layer

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10. Frequently Asked Questions

Can I configure the template for my panel and sample type?

Yes. Every section is editable. Swap in different fluorochrome panels (3-color through 18-color spectral), change the sample source (PBMC, whole blood, dissociated tumor, cell line), adjust the viability dye (Live/Dead Near-IR, Zombie Aqua, 7-AAD, PI), or change the cytometer model and laser configuration. Save your edits as a private template scoped to your lab, or publish back to the ELabELN template library.

Does the template handle compensation tracking?

The compensation matrix is recorded as part of the acquisition metadata. The template captures whether single-color compensation controls were run on the same day, the compensation method used (auto or manual), and any spillover adjustments applied. Linking the FCS export and FlowJo workspace as attachments preserves the full audit trail for the gated analysis.

How does the template handle FMO controls?

FMO (fluorescence-minus-one) control samples are recorded as separate experiments linked to the panel run. The template includes a field for whether FMO boundaries were used and for which markers, supporting the typical immunophenotyping workflow where activation or rare-population markers need an FMO to set the positive-gate threshold.

Is it included in every ELabELN plan?

Yes. Every published template in the ELabELN library is available to all subscribers in both the Standard and Suite editions, with unlimited users in either edition and no per-template fee.

Can my whole lab use this template?

Yes. Every ELabELN edition includes unlimited users, so the PI, postdocs, grad students, rotating researchers, and visiting collaborators can all use the template without per-seat charges. Granular permissions let the PI control who can edit flow runs versus only review them.

How quickly can my lab start running flow in ELabELN?

ELabELN Standard cloud deployment is typically live in one to two business days. Once the instance is provisioned, the template loads from the library in a single click, with the panel, sample source, and acquisition parameters editable to match the cytometer and protocol your lab actually runs.

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11. Conclusion

The ELabELN Flow Cytometry Experiment Template is not simply a digital version of a panel worksheet. It is a structured, audit-ready, searchable record that connects to your antibody and dye inventory, your SOPs and panel validation documents, the sample the panel measured, your cytometer and FlowJo workspace, and the frequency reporting and cohort analysis the results feed — and, in the Suite edition, population-frequency dashboards. It turns a multiparameter immunoassay into a record whose panel, compensation, and gating are captured, comparable, and defensible.

For a laboratory that runs flow regularly, the benefit compounds with every panel. Panel compositions, acquisition settings, and gated frequencies accumulate into one queryable, cross-run QC history. Every antibody lot and titer is traceable. Every SOP and panel version is documented. Every run is timestamped, and every frequency is authenticated — so a lot change or a shifted gate can no longer quietly undermine a cohort result, and drift is caught before it costs a study.

The template is available to all ELabELN subscribers today. A new instance is live in one to two business days, and the first structured panel is typically complete within a week of account creation.

> **GET STARTED**
> Visit elabeln.com/get-started to request your ELabELN instance, or contact LabLynx at 866.522.5969 or info@lablynx.com. Load the flow cytometry template, configure it for your panel and sample type, and run your next panel from a tested, audit-ready baseline.

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